Accurate species identification is crucial to estimate species richness in biodiversity assessments and conservation studies. Morphological identification remains the gold standard but it is time-consuming, prompting for faster, more efficient and cheaper methods. Hence, DNA barcoding was proposed as an alternative. Presently, application of Next-Generation Sequencing technologies allows for thousands of species to be sequenced in parallel. This project applies the technique to the long-legged flies, family Dolichopodidae to achieve comparison of congruence between morphology-based methods and DNA barcoding based on cytochrome c oxidase (COI) gene.

**Project Aims**
- To examine the congruence between morphological-based methods and DNA barcoding for species identification
- To establish barcode database for Dolichopodidae in Singapore with morphological identifications and images
- To assess species diversity across habitats in mangroves and Nee Soon Swamp Forest (freshwater)

**Case Study: Dolichopodidae**
- High species richness discovered in Singapore yet many remain unknown
- Collaboration with leading taxonomist Dr. Grootaert who conducts morphological identifications
- Potential applications as bioindicators of abiotic conditions of wetland habitats and biological control agents

**MATERIALS & METHODS**

1. **Malaise Trap Sampling in Mangroves & Nee Soon**
   - 4701 specimens
   - Figure 1: Hercostomus singapurensis (left) and Neurigona squamifera (right) are two common species found in Singapore.

2. **Direct PCR & Next-Generation Sequencing (NGS)**
   - Sequencing of 313 bp COI gene region
   - Forward and reverse primers were tagged with unique sequences for individualised tracking
   - Table 1: Success rates of NGS for DNA barcoding of COI gene

3. **Data Analysis: Identification of Species Unit**
   - COI sequences were clustered by pairwise distance values based on nucleotide similarities
   - Putative species units are inferred from molecular operational taxonomic units (MOTUs)

4. **Results & Discussion**
   - 162 species were found in Singapore from 3221 specimens
     - 1744 specimens were pre-identified (due to novel identified and described)
     - Combined MOTU clustering showed 162 species in mangroves and Nee Soon
     - Extensive database with COI barcodes, morphological identifications and high-resolution images
     - Online database at Animal and Plants of Singapore (APS) [http://nathist.science.nus.edu.sg/#]

**ACKNOWLEDGMENTS**

- Prof. Meier for his patient guidance and supervision of this project and enriching learning experience
- Wendy, Amtita, Darren, Mao Sheng, Nicholas and other NUS Evolab members for their guidance
- Dr. Grootaert for his significant contributions to morphological identifications of Dolichopodidae

**REFERENCES**


**CONCLUSIONS & FUTURE WORK**

- DNA barcoding allows rapid species identification and discovery.
- High congruence and stability of species units count to estimate species richness
- New species discoveries added to an extensive database for Dolichopodidae
- Applicability to pre-sorting of specimens to aid future ecological and conservation studies
- No species overlap in mangroves and Nee Soon

To improve habitat sampling and assessment of geographic and temporal variations